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The cardioprotective effects of nitroglycerin-induced preconditioning are mediated by calcitonin gene-related peptide

Chang-Ping Hu, Yuan-Jian Li *, Han-Wu Deng

Department of Pharmacology, Hunan Medical University, Changsha, Hunan 410078, China

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Abstract

Previous investigations have shown that endogenous calcitonin gene-related peptide (CGRP) may play an important role in the mediation of ischemic preconditioning and that nitroglycerin evokes the release of CGRP. In the present study, we examined whether nitroglycerin provides a preconditioning stimulus, and whether the cardioprotective effects of nitroglycerin-induced preconditioning involve endogenous CGRP. Thirty minutes of global ischemia and 30 min of reperfusion caused a significant impairment of cardiac contractile function and an increased release of creatine kinase. Pretreatment with nitroglycerin at the concentration of 3×10^{-7} or 10^{-6} M for 5 min produced a significant improvement of cardiac function and a decrease in the release of creatine kinase. The content of CGRP-like immunoreactivity in coronary effluent was increased during nitroglycerin perfusion. However, the cardioprotection afforded by nitroglycerin was abolished by CGRP-(8-37) (10^{-7} M), a selective CGRP receptor antagonist. Pretreatment with capsaicin (50 mg/kg, s.c.), which specifically depletes the transmitter content of sensory nerves, also abolished the protective effects of nitroglycerin and markedly reduced the release of CGRP from the heart during nitroglycerin perfusion. These findings suggest that nitroglycerin-induced preconditioning is related to stimulation of CGRP release in rat hearts.

Keywords: Nitroglycerin; Preconditioning; CGRP (calcitonin gene-related peptide); CGRP-(8-37); Capsaicin; Heart, rat

1. Introduction

Ischemic preconditioning has been defined as the tolerance of the myocardium to subsequent sustained ischemic damage after the heart has been subjected to one or more brief periods of ischemic stress (Murry et al., 1986). The mechanism responsible for preconditioning has not been fully elucidated. There is a growing amount of evidence that endogenous myocardial protective substances may play a pivotal role in ischemic preconditioning (Parratt, 1993, 1994).

Calcitonin gene-related peptide (CGRP) is a principal transmitter of sensory nerves and is present in the hearts of animals and humans (Wharton et al., 1986; Franco-Cereceda, 1988). We have recently suggested that CGRP may be an endogenous myocardial protective substance and may play an important role in the mediation of ischemic preconditioning in isolated perfused rat hearts (Li et al., 1996; Peng et al., 1996; Xiao et al., 1996).

Nitroglycerin relieves angina by causing vasodilatation. It has recently been found that nitroglycerin evokes the release of CGRP from vascular tissues in both the central nervous system and the periphery (Wei et al., 1992; Fanciullacci et al., 1995; Booth et al., 1997). Because of the possible mediation of CGRP in ischemic preconditioning and CGRP release stimulated by nitroglycerin, in the present study we examined whether the protective effects of nitroglycerin-induced preconditioning involve endogenous CGRP in isolated rat hearts.

2. Materials and methods

2.1. Heart preparation

Sprague–Dawley male rats weighing 200–250 g were anesthetized by intraperitoneal administration of 60 mg/kg sodium pentobarbital. The hearts were rapidly excised and placed in ice-cold Krebs–Henseleit buffer solution containing NaCl 119.0, $NaHCO_3$ 25.5, KCl 4.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.0 mM. The heart

* Corresponding author. Tel.: +86-731-4474411 ext. 2704; Fax: +86-731-4471339

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Table 1
Effect of nitroglycerin on cardiac function during reperfusion

	n	Preischemia	Reperfusion (min)			
			5	10	20	30
Left ventricular pressure (mm Hg)						
Control	5	82.7 ± 2.6	79.3 ± 2.3	78.2 ± 2.7	77.0 ± 2.1	75.0 ± 1.3
Ischemia/Reperfusion	6	82.0 ± 2.9	33.2 ± 2.8[b]	30.8 ± 2.7[b]	37.4 ± 3.9[b]	38.6 ± 3.1[b]
+ Nitroglycerin (10^{-7} M)	5	79.5 ± 2.2	19.5 ± 5.4	25.2 ± 5.3	31.1 ± 5.6	33.7 ± 5.7
+ Nitroglycerin (3×10^{-7} M)	5	77.1 ± 4.1	73.4 ± 12.5[d]	84.9 ± 6.6[d]	84.0 ± 6.8[d]	76.8 ± 7.6[d]
+ Nitroglycerin (10^{-6} M)	7	77.6 ± 2.5	41.4 ± 6.0	61.9 ± 6.2[d]	68.5 ± 4.0[d]	73.3 ± 3.1[d]
$+dp/dt_{max}$ (mm Hg/s)						
Control	5	2667 ± 40	2668 ± 96	2672 ± 105	2588 ± 83	2500 ± 35
Ischemia/Reperfusion	6	2832 ± 105	674 ± 60[b]	676 ± 81[b]	928 ± 134[b]	1034 ± 106[b]
+ Nitroglycerin (10^{-7} M)	5	2754 ± 76	396 ± 122	557 ± 140	774 ± 166	831 ± 151
+ Nitroglycerin (3×10^{-7} M)	5	2514 ± 136	1855 ± 365[d]	2268 ± 209[d]	2534 ± 227[d]	2511 ± 260[d]
+ Nitroglycerin (10^{-6} M)	7	2492 ± 87	959 ± 112	1268 ± 202[c]	1729 ± 139[d]	2028 ± 97[d]
$-dp/dt_{max}$ (mm Hg/s)						
Control	5	1796 ± 21	1630 ± 98	1605 ± 90	1553 ± 56	1490 ± 50
Ischemia/Reperfusion	6	1982 ± 38	459 ± 22[b]	528 ± 84[b]	761 ± 122[b]	824 ± 93[b]
+ Nitroglycerin (10^{-7} M)	5	2201 ± 165	299 ± 83	460 ± 122	628 ± 127	689 ± 124
+ Nitroglycerin (3×10^{-7} M)	5	1916 ± 184	1322 ± 218[d]	1635 ± 105[d]	1810 ± 148[d]	1733 ± 169[d]
+ Nitroglycerin (10^{-6} M)	7	1928 ± 96	843 ± 113[c]	1151 ± 201[d]	1456 ± 97[d]	1566 ± 55[d]
Coronary flow (ml/min)						
Control	5	9.4 ± 0.2	9.0 ± 0.1	9.1 ± 0.1	8.8 ± 0.1	8.8 ± 0.1
Ischemia/Reperfusion	6	9.6 ± 0.6	4.8 ± 0.3[b]	4.4 ± 0.2[b]	4.2 ± 0.2[b]	3.8 ± 0.2[b]
+ Nitroglycerin (10^{-7} M)	5	10.6 ± 1.0	3.7 ± 0.4	3.9 ± 0.4	3.9 ± 0.4	3.8 ± 0.4
+ Nitroglycerin (3×10^{-7} M)	5	9.4 ± 0.6	9.6 ± 0.9[d]	9.4 ± 0.6[d]	8.6 ± 0.5[d]	8.1 ± 0.6[d]
+ Nitroglycerin (10^{-6} M)	7	9.6 ± 0.4	9.1 ± 0.4[d]	8.9 ± 0.4[d]	8.7 ± 0.3[d]	8.6 ± 0.2[d]
Heart rate (beats/min)						
Control	5	292 ± 12	285 ± 12	297 ± 13	288 ± 14	296 ± 18
Ischemia/Reperfusion	6	313 ± 12	159 ± 44[a]	204 ± 24[a]	235 ± 13	255 ± 17
+ Nitroglycerin (10^{-7} M)	5	310 ± 14	165 ± 29	191 ± 33	202 ± 28	225 ± 22
+ Nitroglycerin (3×10^{-7} M)	5	295 ± 15	280 ± 21[c]	277 ± 20[c]	279 ± 18	305 ± 8
+ Nitroglycerin (10^{-6} M)	7	305 ± 7	262 ± 11[c]	268 ± 8[c]	261 ± 7	270 ± 6

[a] $P < 0.05$ and [b] $P < 0.01$ vs. control, [c] $P < 0.05$ and [d] $P < 0.01$ vs. ischemia/reperfusion.

was attached to a Langendorff apparatus via the aorta for retrograde perfusion with Krebs–Henseleit buffer solution (Srimani et al., 1990). The perfusate was equilibrated with 95% O_2 and 5% CO_2, maintained at 37°C and pH 7.4. Perfusion pressure was maintained at 85 cm H_2O. A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle via the mitral valve to record isovolumic left ventricular pressure (Qiu and Hearse, 1992). Left ventricular pressure, the first derivative of left ventricular pressure ($\pm dp/dt_{max}$), and heart rate were continuously monitored. The resulting electric signals were digitized by a MacLab analogue-to-digital converter and recorded by a Power Macintosh 7220 computer. Coronary flow was measured by timed collection of coronary effluent and samples of coronary effluent after 5 min of reperfusion were collected for measurement of creatine kinase.

2.2. Creatine kinase measurement

The creatine kinase activity in the coronary effluent was measured spectrophotometrically. Supplies for the creatine kinase assay were obtained from Beijing Zhongsheng High-tech Bioengineering, Beijing, China.

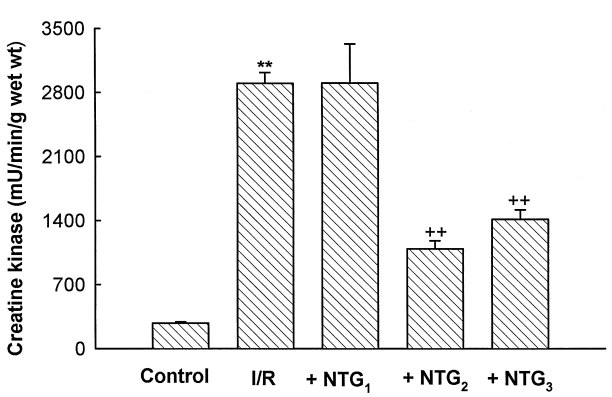

Fig. 1. Effect of nitroglycerin (NTG) on creatine kinase release during reperfusion. I/R: ischemia–reperfusion; NTG_1, NTG_2 and NTG_3: preparations were perfused with nitroglycerin at the concentration of 10^{-7}, 3×10^{-7} or 10^{-6} M, for 5 min. Values are means ± S.E.M. ($n = 5$–7). $^{**}P < 0.01$ vs. control; $^{++}P < 0.01$ vs. I/R.

2.3. Radioimmunoassay

Perfusate fractions (5 min) were collected in acetic acid (final concentration 0.2 M), desalted using SEP-PAK C_{18} cartridges and lyophilized. CGRP-like immunoreactivity in the perfusate fraction was measured by using a radioimmunoassay kit with antisera raised against rat CGRP, ^{125}I-labelled CGRP and rat CGRP standard.

2.4. Experimental protocols

All hearts, except those of the control group, were allowed an initial 20-min stabilization period before being subjected to 30 min of global ischemia and 30 min of reperfusion.

Five groups of animals were studied to evaluate the cardioprotective effects of pretreatment with nitroglycerin. The control group was perfused with Krebs–Henseleit buffer solution throughout the experiment. The ischemia–reperfusion group underwent 30 min of ischemia and 30 min of reperfusion. For the nitroglycerin-treated groups, the hearts were perfused with nitroglycerin at the concentration of 10^{-7}, 3×10^{-7} or 10^{-6} M for 5 min and then washed out for 10 min with nitroglycerin-free Krebs–Henseleit buffer solution before the 30-min ischemic period.

A second series of experiments was designed to evaluate the role of CGRP in nitroglycerin-induced preconditioning. For the studies on the effect of capsaicin on the protection provided by nitroglycerin, preparations were

Table 2
Effect of capsaicin or CGRP-(8-37) on the effect of nitroglycerin on cardiac function during reperfusion

	n	Preischemia	Reperfusion (min)			
			5	10	20	30
Left ventricular pressure (mm Hg)						
Control	5	93.0 ± 5.6	89.3 ± 4.9	86.7 ± 4.6	85.2 ± 4.6	83.6 ± 3.7
Ischemia/Reperfusion	5	89.6 ± 4.8	17.0 ± 2.2[b]	22.4 ± 2.7[b]	28.7 ± 3.1[b]	34.2 ± 4.8[b]
+ Nitroglycerin (NTG)	6	88.0 ± 5.5	50.0 ± 3.9[d]	66.2 ± 5.6[d]	68.6 ± 4.7[d]	64.0 ± 5.0[d]
+ Vehicle and NTG	7	88.4 ± 5.6	58.4 ± 4.1	75.0 ± 8.5	73.8 ± 7.0	69.4 ± 5.7
+ Capsaicin and NTG	6	92.4 ± 6.4	16.2 ± 4.9[f]	26.7 ± 4.7[f]	40.8 ± 5.4[f]	48.7 ± 5.6[f]
+ $CGRP_{8-37}$ and NTG	5	82.1 ± 2.3	22.7 ± 4.5[h]	32.1 ± 6.1[h]	38.6 ± 6.1[h]	43.1 ± 4.8[g]
+ dp / dt$_{max}$ (mm Hg / s)						
Control	5	3064 ± 180	2944 ± 168	2874 ± 161	2829 ± 173	2775 ± 178
Ischemia/Reperfusion	5	2611 ± 213	418 ± 68[b]	553 ± 57[b]	743 ± 62[b]	935 ± 130[b]
+ Nitroglycerin (NTG)	6	2773 ± 233	1387 ± 142[d]	1989 ± 187[d]	2162 ± 130[d]	2088 ± 125[d]
+ Vehicle and NTG	7	2920 ± 190	1666 ± 128	2281 ± 206	2347 ± 173	2271 ± 157
+ Capsaicin and NTG	6	2831 ± 146	276 ± 80[f]	585 ± 112[f]	1048 ± 185[f]	1338 ± 192[f]
+ $CGRP_{8-37}$ and NTG	5	2641 ± 58	456 ± 96[h]	773 ± 157[h]	1054 ± 207[h]	1217 ± 181[h]
− dp / dt$_{max}$ (mm Hg / s)						
Control	5	2035 ± 143	2017 ± 152	1943 ± 145	1919 ± 154	1810 ± 118
Ischemia/Reperfusion	5	1896 ± 143	304 ± 58[b]	402 ± 54[b]	533 ± 58[b]	640 ± 99[b]
+ Nitroglycerin (NTG)	6	1905 ± 121	1069 ± 102[d]	1448 ± 134[d]	1517 ± 110[d]	1406 ± 103[d]
+ Vehicle and NTG	7	1881 ± 147	1149 ± 72	1587 ± 142	1539 ± 107	1472 ± 95
+ Capsaicin and NTG	6	1959 ± 143	245 ± 78[f]	444 ± 83[f]	783 ± 119[f]	950 ± 115[f]
+ $CGRP_{8-37}$ and NTG	5	2040 ± 88	353 ± 76[h]	614 ± 117[h]	835 ± 147[h]	958 ± 104[h]
Coronary flow (ml / min)						
Control	5	9.5 ± 0.2	9.4 ± 0.3	9.3 ± 0.2	9.2 ± 0.3	9.1 ± 0.2
Ischemia/Reperfusion	5	11.0 ± 0.7	4.4 ± 0.2[b]	4.4 ± 0.1[b]	4.7 ± 0.1[b]	4.5 ± 0.2[b]
+ Nitroglycerin (NTG)	6	9.9 ± 0.8	8.6 ± 0.8[d]	9.1 ± 0.9[d]	8.7 ± 0.6[d]	8.1 ± 0.6[d]
+ Vehicle and NTG	7	11.7 ± 0.6	10.0 ± 1.2	10.8 ± 1.3	10.7 ± 1.2	10.3 ± 1.2
+ Capsaicin and NTG	6	11.1 ± 0.6	5.1 ± 0.3[f]	5.4 ± 0.4[f]	5.5 ± 0.5[f]	5.4 ± 0.5[f]
+ $CGRP_{8-37}$ and NTG	5	9.9 ± 0.8	4.2 ± 0.2[h]	4.2 ± 0.3[h]	4.3 ± 0.2[h]	4.1 ± 0.1[h]
Heart rate (beats / min)						
Control	5	300 ± 11	295 ± 10	293 ± 10	292 ± 9	285 ± 10
Ischemia/Reperfusion	5	318 ± 17	206 ± 39[a]	221 ± 30[a]	262 ± 31	285 ± 20
+ Nitroglycerin (NTG)	6	314 ± 15	308 ± 10[c]	296 ± 13[c]	302 ± 13	317 ± 16
+ Vehicle and NTG	7	315 ± 16	313 ± 10	305 ± 12	292 ± 13	287 ± 12
+ Capsaicin and NTG	6	280 ± 11	177 ± 26[e]	233 ± 25	236 ± 27	253 ± 20
+ $CGRP_{8-37}$ and NTG	5	315 ± 9	185 ± 54[g]	259 ± 27	280 ± 21	277 ± 13

[a] $P < 0.05$ and [b] $P < 0.01$ vs. control, [c] $P < 0.05$ and [d] $P < 0.01$ vs. ischemia/reperfusion, [e] $P < 0.05$ and [f] $P < 0.01$ vs. vehicle and NTG, [g] $P < 0.05$ and [h] $P < 0.01$ vs. nitroglycerin.

exposed to nitroglycerin (3×10^{-7} M) after pretreatment with capsaicin. Capsaicin (50 mg/kg) or vehicle was administrated by s.c. injection 4 days before the experiments. In the case of CGRP-(8-37), preparations were exposed to CGRP-(8-37) (10^{-7} M) for 5 min, then CGRP-(8-37) and nitroglycerin (3×10^{-7} M) together for 5 min, followed by a 10-min wash-out period before the 30-min ischemic period.

2.5. Reagents

CGRP-(8-37) and capsaicin were purchased from Sigma. Nitroglycerin was purchased from Beijing Yiming Pharmaceutical Factory, Beijing, China. Capsaicin was dissolved in a vehicle containing 10% Tween 80, 10% ethanol, and 80% saline. The radioimmunoassay kit used was obtained from Dongya Immunity Technology Institution, Beijing, China.

2.6. Statistics

All values are expressed as means ± S.E.M. One-way analysis of variance combined with the Newman–Keuls test was used to test group differences in cardiac function, release of creatine kinase, and content of CGRP-like immunoreactivity. Paired *t*-test was used for within-group analysis to test differences in the content of CGRP-like immunoreactivity. A *P*-value of less than 0.05 was considered significant.

3. Results

3.1. Effects of nitroglycerin

In the control group, continuously perfused rat hearts were monitored for 90 min. There were no significant changes in heart rate, coronary flow, left ventricular pressure, and $\pm dp/dt_{max}$. Thirty minutes of global ischemia and 30 min of reperfusion caused a decrease in cardiac function (heart rate, coronary flow, left ventricular pressure, and $\pm dp/dt_{max}$) and a significant increase in the release of creatine kinase during reperfusion (Table 1; Fig. 1).

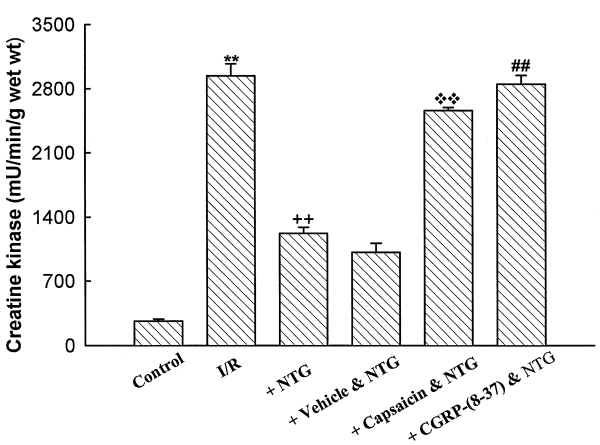

Fig. 2. Influence of capsaicin or CGRP-(8-37) on the effect of nitroglycerin (NTG) on creatine kinase release during reperfusion. I/R: ischemia–reperfusion; NTG: preparations were exposed to nitroglycerin (3×10^{-7} M) for 5 min. Capsaicin or vehicle was administrated by s.c. injection 4 days before perfusion with nitroglycerin. Values are means ± S.E.M. ($n = 5$–7). $^{**}P < 0.01$ vs. control; $^{++}P < 0.01$ vs. I/R; $^{\blacklozenge\blacklozenge}P < 0.01$ vs. +vehicle and NTG; $^{\#\#}P < 0.01$ vs. +NTG.

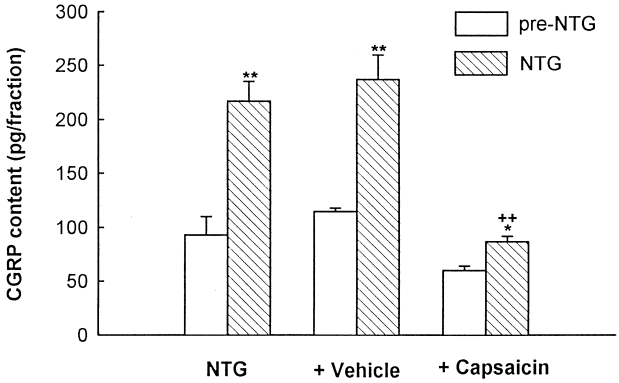

Fig. 3. Effect of nitroglycerin (NTG) on the release of CGRP. NTG: preparations were exposed to nitroglycerin (3×10^{-7} M) for 5 min. Capsaicin or vehicle was administrated by s.c. injection 4 days before perfusion with nitroglycerin. Values are means ± S.E.M. ($n = 5$–6). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. pre-NTG; $^{++}P < 0.01$ vs. NTG or +vehicle.

Pretreatment with nitroglycerin at the concentration of 3×10^{-7} or 10^{-6} M for 5 min caused a significant improvement of cardiac function and a decrease in the release of creatine kinase during reperfusion. However, nitroglycerin at the concentration of 10^{-7} M had no effect on reperfusion-induced cardiac dysfunction and creatine kinase release (Table 1; Fig. 1).

3.2. Effect of CGRP-(8-37) or capsaicin on the cardioprotection provided by nitroglycerin

To test whether the protective effects of nitroglycerin-induced preconditioning involve the activation of sensory nerves, capsaicin was used. After pretreatment with capsaicin to deplete the transmitter of sensory nerves, the cardioprotective effects of nitroglycerin were abolished (Table 2; Fig. 2).

To further explore the mediation of CGRP in the protective effects of nitroglycerin, CGRP-(8-37) was used. In the presence of CGRP-(8-37), a selective CGRP receptor antagonist, the protective effects of nitroglycerin were also abolished (Table 2; Fig. 2).

As shown in Fig. 3, the content of CGRP-like immunoreactivity in coronary effluent was significantly increased during nitroglycerin perfusion. However, after pretreatment with capsaicin, nitroglycerin only caused a slight increase in the effluent content of CGRP-like immunoreactivity. Levels of CGRP-like immunoreactivity in the coronary effluent in the capsaicin plus nitroglycerin-treated

group were significantly lower than those of nitroglycerin-treated group.

4. Discussion

The major new findings of the present study are: (1) a 5-min perfusion of nitroglycerin reduces reperfusion-induced cardiac dysfunction and decreases the elevation of creatine kinase release during reperfusion in isolated rat hearts, and (2) nitroglycerin produces its cardioprotective effects via stimulation of CGRP release, because nitroglycerin caused an increase in the level of CGRP-like immunoreactivity in coronary effluent, and because the protective effects of nitroglycerin were abolished by CGRP-(8-37) or capsaicin.

Nitroglycerin remains one of the most useful drugs in the short-term management of acute ischemic coronary syndromes. In addition to its well-established venodilating activity, nitroglycerin is now known to cause vasorelaxation of coronary arteries, coronary stenoses, and coronary collateral vessels and to prevent episodic coronary constriction (Abrams, 1995). The vasodilator response to nitroglycerin is ascribed to the release of nitric oxide (Abrams, 1995; Katzung and Chatterjee, 1995). Recently, it has been found that nitroglycerin activates sensory nerve fibers to release CGRP from vascular tissues in both the central nervous system and the periphery (Wei et al., 1992; Fanciullacci et al., 1995; Booth et al., 1997). Based on previous observations that CGRP, either endogenous or exogenous, protects the myocardium against ischemia–reperfusion injury (Xiao et al., 1996; Li et al., 1996), it is probable that nitroglycerin induces preconditioning-like cardioprotection. In the present study, pretreatment with nitroglycerin (3×10^{-7} or 10^{-6} M) for 5 min significantly attenuated the myocardial damage caused by ischemia–reperfusion, as shown by the improved recovery of cardiac function and the decreased release of creatine kinase. These results suggest that nitroglycerin may provide a preconditioning stimulus.

The mechanism responsible for the cardioprotective effects of nitroglycerin-induced preconditioning is unclear. A consistent and convincing body of evidence has suggested that the cardioprotective effects of ischemic preconditioning are mediated by endogenous chemical mediators (Parratt, 1993, 1994). As mentioned above, the beneficial effects of ischemic preconditioning are due to CGRP release stimulated by ischemia (Xiao et al., 1996). Direct exposure of the heart to capsaicin, to stimulate capsaicin-sensitive sensory nerves, reduces myocardial injury induced by ischemia–reperfusion in isolated rat hearts (Li et al., 1996). Others have reported that the in vivo cardioprotective effect of pacing-induced preconditioning is abolished by pretreatment with capsaicin, which depletes the transmitter content of sensory nerves (Ferdinandy et al., 1997). Recently, we have shown that the cardioprotection provided by bradykinin-induced preconditioning is mediated by endogenous CGRP (Song et al., 1999). In the present study, the cardioprotective effects of nitroglycerin were also abolished by pretreatment with capsaicin, suggesting that the effects of nitroglycerin are related to the activation of capsaicin-sensitive sensory nerves. These findings suggest that various factors that stimulate capsaicin-sensitive sensory nerves may induce protection of the myocardium.

To further test whether CGRP is involved in the mediation of nitroglycerin-induced preconditioning, CGRP-(8-37) was used. Our results revealed that the protective effects of nitroglycerin were abolished in the presence of CGRP-(8-37), a selective CGRP receptor antagonist. Previous investigations have shown that the cardioprotective effects of ischemic preconditioning are abolished by CGRP-(8-37) (Peng et al., 1996; Xiao et al., 1996). These results suggest that CGRP may be an endogenous myocardial protective substance.

In the present study, we also tested the effects of nitroglycerin on CGRP release in cardiac sensory nerves. The results showed that nitroglycerin perfusion caused a significant improvement of cardiac function and a decrease in the release of creatine kinase concomitantly with an increase in the content of CGRP-like immunoreactivity in the coronary effluent. However, after pretreatment with capsaicin to deplete transmitters in sensory nerves, the level of CGRP-like immunoreactivity was significantly reduced and the myocardial protection afforded by nitroglycerin was also abolished. This supports the hypothesis that the protective effects of nitroglycerin are due to the stimulation of CGRP release.

It is noteworthy that the protective effects of nitroglycerin-induced preconditioning appeared to be concentration-independent. A similar effect has also been seen for a variety of drugs (Lasley and Mentzer, 1992; Mosca et al., 1994; Tosaki et al., 1995). It has been suggested that ischemic or pharmacological preconditioning protects against myocardial injury by triggering signal transduction pathways (Parratt, 1994). It is likely that the effects of drugs will not increase with increasing dosage once endogenous protective mechanisms have been triggered.

The mechanism by which nitroglycerin stimulates the release of CGRP from sensory nerve fibers is not known. It has been shown that the vasorelaxation produced by nitroglycerin is mediated by nitric oxide as well as prostaglandins (Katzung and Chatterjee, 1995). There is evidence to suggest that both endogenous nitric oxide and prostaglandins are capable of modulating sensory neurotransmission (Franco-Cereceda et al., 1994; Hughes and Brain, 1994). However, the exact mechanism of the release of CGRP induced by nitroglycerin requires further investigation.

In summary, the present results suggest that nitroglycerin induces preconditioning-like cardioprotection. They

also suggest that the effects of nitroglycerin are due to stimulation of endogenous CGRP release in rat hearts.

Acknowledgements

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